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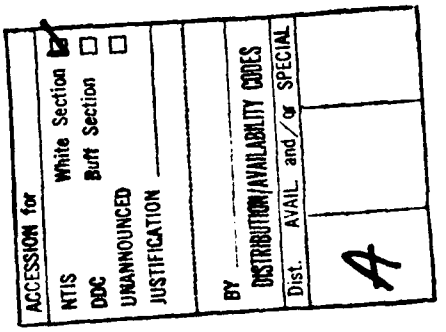

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AN ACCELERATED METHOD FOR STAINING TULAREMIA BACTERIA

/Article by R.I. Kudelina, Tularemia Laboratory/ Institute of Epidemiology and Microbiology imeni N.F. Gamaleya/ USSR Academy of Medical Sciences/ Moscow, Laboratornoye Delo, № 4, 1974, p 250, /

At the present time the standard procedure for staining tularemia bacteria in animal tissue is the staining of the smear-impression according to Romanovskiy-Gimze. During the cultivation of tularemia strains in the organism of chick embryos we came up against the fact that this stain makes it difficult to differentiate tularemia bacteria in the smears of vitelline sacs. The duration of the staining procedure, from several hours to days and the need to fix the smear also create difficulties in its application.

In a search for a more optimal method we decided on the method of accelerate staining using the Gimze solution, which is employed in staining the blood of farm animals /1/. This method has not been previously employed to stain the tularemia microbe, and literature available to us did not provide references thereto.

The advantage of the method over others is found in the rapidity of the staining procedure (15 min). Moreover, preliminary fixing is not required since the staining and fixing of the smear are carried out simultaneously.

The accelerated staining of tularemia bacteria calls for a mixing of equal parts of the Romanovskiy-Gimze stain and methyl alcohol or chemically pure acetone. This mixture enclosed in vials with ground stoppers may be kept for several months.

In order to use single smears we use Petri dishes with a closed cover, and for a staining of a large number of smears we use ice trays (without the screen) contained in each freezer.

The staining technology is as follows: a fresh (not less than 24 hour old) air dried and unfixed smear is covered with 20 drops of the mixture of the Romanovskiy-Gimze stain and methyl alcohol[1]. after 1 minute we add to the stain 10 ml of alkalized (pH7.2) distilled water. Carefully mixe the mixture with water. After 10-15 minutes the water and the mixture are combined, the smear is washed with running water, dried and examined under a microscope.

Using this method of staining the tularemia bacteria we have a dark-violet color and is clearly differentiated from the surrounding light-lilac celular background.

This method described by us was tested during the staining of the smears-stains (over 2,000) from vitelline sacs of chick embryos and organs of white rats, guinea pigs infected with tularemia microbes. In addition we obtained good results allowing us to recomend this method for practical use.

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